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Stability Evaluation of Methylparaben and Propylparaben in their Solution Aqua Conservans Using HPLC

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Abstract

HPLC was used to determine the content of methylparaben (MP), propylparaben (PP) and 4-hydroxybenzoic acid (4-HYB) as their decomposition product in the water solution Aqua Conservans (AQCO) in accordance with the Czech Pharmacopoeia and the Slovak Pharmaceutical Codex (0.067 % MP and 0.033 % PP). Based on the results of the analyses of 10 samples of AQCO from 8 pharmacies, 4-42 months after their preparation, an expiration period of 3 years was proposed for AQCO.

Keywords

parabens, Aqua Conservans, stability, 4-hydroxybenzoic acid, HPLC

Introduction

Aqueous solutions of parabens (alkylesters of 4-HYB) are used in pharmaceutical technology for their antimicrobial and antifungal effect. A typical example of these is Aqua Conservans (AQCO), mentioned in some pharmacopoeias, e.g. the Czech Pharmacopoeia [1] and pharmaceutical codexes, e.g. NRF [2] and the forthcoming Slovak Pharmaceutical Codex 2007 [3]. AQCO in accordance with the Czech Pharmacopoeia and the Slovak Pharmaceutical Codex 2007 contains 0.067 % MP and 0.033 % PP, while in accordance with NRF [2] it

contains 0.075 % MP and 0.025 % PP. NRF [2] prescribes a 2-year expiration period for AQCO. The directive of the State Institute for Drug Control in Prague [4] is valid for the solution in accordance with the Czech Pharmacopoeia [1], which prescribes an expiration period of 3 months only for AQCO. Due to the big difference between these two data, the stability of parabens in the solution in accordance with the Czech Pharmacopoeia was observed by spectrophotometry in ultraviolet (parabens) and visible (4-HYB) regions [5]. The results of the analyses of samples 3.5 to 10.5 months after their preparation implied a longer expiration period than that recommended by the directive [4]. The aim of this paper is to evaluate the hydrolytic stability of AQCO (decomposition of parabens to 4-HYB) with a composition which is in accordance with the Czech Pharmacopoeia when storing for a longer period. HPLC was chosen as a suitable analytical method, which was successful in determination the parabens and 4-HYB in medicinal preparations and cosmetics [6-8].

Results and Discussion

A methanol-water mixture 60:40 v/v was used as a suitable mobile phase for 4-HYB, MP and PP separation on the Lichrospher RP18 column [6], but when it was used on the Kromasil® C18 7.5μm column, the retention time of PP was too long. The desirable retention of all the analytes (4-HYB, MP, PP) was acquired by changing the composition of the mobile phase to methanol-water-acetic acid 69:30:1 v/v/v. Under the conditions given in Experimental, the retention time of PP as the analyte last eluted from the column did not exceed 6 minutes. The resolution between 4-HYB and MP peaks was 3.8 and between MP and PP 8.8 (arithmetic mean from n = 10), see Fig.1.

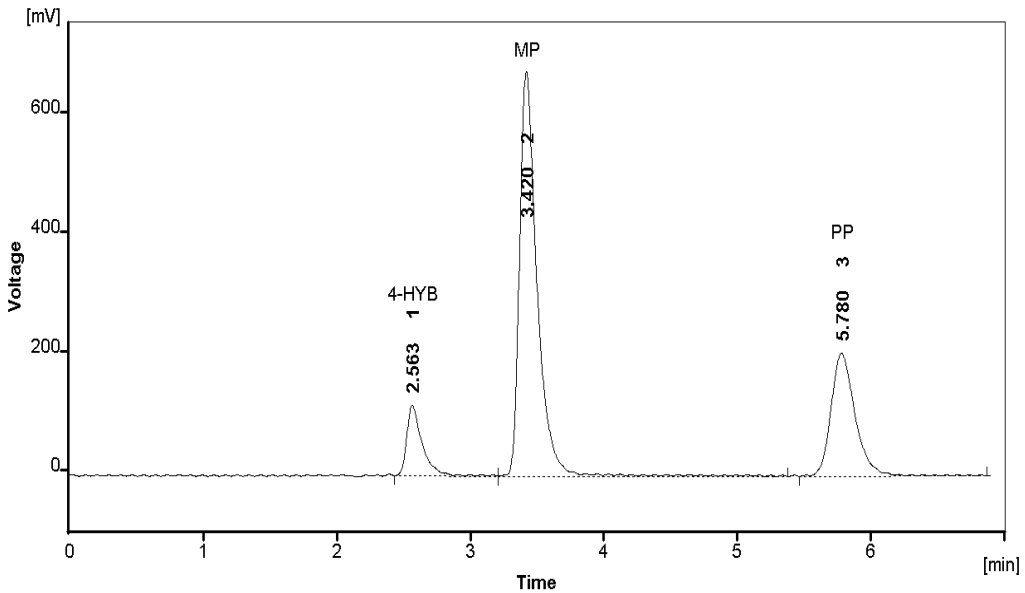

Fig.1. A chromatogram of the mixture containing 1.7 × 10^{-4} % 4-HYB, 12 × 10^{-4} % MP and 6.0 × 10^{-4} % PP (see Experimental for detailed conditions).

After confirming the linearity of the dependence of analytical signals on analyte concentration in the satisfactory range (0.13 × 10^{-4} – 16 × 10^{-4} %, n = 14, linear correlation coefficient r ≥ 0,9996), quantitative analyses of AQCO samples of various ages were performed by comparing directly with one standard solution, which contained all the three determined analytes. The validation of the peak heights gave results for all the analytes, whose arithmetic mean was in better agreement with theory than that from peak areas (the difference for all the analytes was lower than 1.0%). Similarly, the results for relative standard deviations of analytes determination from the peak heights were lower than from peak areas (values were lower than 2.0%). Therefore the evaluation from peak heights was also used for analyses of AQCO samples. Results of their analyses expressed as a percentage of parabens decomposition are summarized in Table 1. The limit of detection (S/N = 3) for 4-HYB was 4.2 x 10^{-5} %.

Sample code*	Sample age (months)	Parabens decomposition (%) **
A1	39.0	3.7
	42.0	4.1
A2	38.5	2.8
	41.5	3.1
A3	4.0	***
	8.0	0.39
B	33.0	12.0****
	36.0	14.2****
C	32.0	11.6
	35.0	13.6****
D	11.5	0.68
E	11.0	0.57****
	13.0	0.75****
F	10.0	0.65****
	12.0	0.71
	13.0	0.73****
	17.0	1.0****
G	4.0	***
	8.0	0.44****
H	4.0	***
	8.0	0.43

Table 1 Decomposition of parabens to 4-HYB in Aqua Conservans.

* A – H were samples from various pharmacies, A1 – A3 were samples from the same pharmacy, but with different date of preparation

** arithmetic mean from 4 assays except ****, where it is mean from 3 assays

*** decomposition was under the detection limit

The decomposition of parabens in most samples is evident to be low. Samples B and C, the decomposition of which was clearly higher, were additionally found to have a pH range between 7 and 9, and not between 6.2 and 6.8, as the randomly selected three other samples. This anomaly was probably caused by a technological error in the preparation of samples B and C, and therefore these samples were omitted as outliers. For samples A1 to A3 and D to H, a dependence of parabens decomposition (y) on a storage period of AQCO (x) can be expressed by the equation $y = 0.09469x – 0.4053$, with the correlation coefficient $r = 0.9839$. This equation tells us that for samples A1 - A3 and D – H, the decomposition of parabens is expected to be only 1.9% if they are stored for 24 months and 3.0% if they are stored for 36 months. This value is lower than the decomposition of ≤5% mentioned in NRF [2] in relation to the expiration period of AQCO with a slightly different composition. Therefore, it is possible to propose an expiration period of 3 years for AQCO in the composition of Czech Pharmacopoeia [1] in view of the stability of the ester bond of parabens. For this, the storing of this solution at a temperature higher than 15°C must be guaranteed in accordance with the demand of NRF [2]. It is due to an insufficient solubility of parabens at a lower temperature.

Experimental

Samples of AQCO prepared in pharmacies were stored in 50mL – 100mL brown glass bottles closed with plastic screw closures. The temperature during their storage ranged between 19 and 26°C. Methanol and acetic acid (Lach-Ner, Neratovice, Czech Republic) were of analytical grade, MP and PP were of a quality required by the Czech Pharmacopoeia, 4-HYB (Fluka) was of puriss grade. The HPLC device (Ecom, Prague) consisted of an LCP 4020 pump (Ecom, Prague), a Rheodyne manual sample injector equipped with 20μL loop, a chromatographic column Kromasil® 100-7-C18 150 x 4.6mm, particle size 7.5μm (Prochrome), maintained at an ambient temperature, and an LCD 2083 spectrophotometric detector (Ecom, Prague). A suitable flow rate for the mobile

phase (methanol-water-acetic acid 69:30:1 v/v/v) was 0.8mL/min. A wavelength of 255nm chosen for spectrophotometric detection conforms to an absorption maximum of 4-HYB in the mobile phase. The injection volume was 20μL. Samples of AQCO were diluted 100times before the analyses in the mobile phase. The chromatographic analyses were evaluated using Clarity Lite 2.1 software (Data Apex, Prague). The chromatographic system was validated by an analysis of solutions with a known analyte concentration equal to a 10% decomposition of parabens to 4-HYB. Both the validation results and the results of the analyses of the AQCO samples were statistically processed by testing for outliers in range, calculation of arithmetic mean and estimation of relative standard deviation [9]. A digital 691 Metrohm pH meter (Switzerland) with a combined Metrohm 6.0218.010 glass electrode was used for pH measurement.

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